Structure-Activity Relationship Study of a Series of *N*-Substituted Piperazinyl-Fluoroquinolones as Anti-*Helicobacter pylori* Agents

A. Foroumadi[a,*], M. Safavi[a], S. Emami[b], F. Siavoshi[c], S. Najjari[a], F. Safari[c] and A. Shafiee[a]

[a]*Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14174, Iran;* [b]*Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran;* [c]*Microbiology Department, Faculty of Sciences, University of Tehran, Tehran, Iran*

Abstract: *Helicobacter pylori* is now recognized as the primary etiological factor associated with gastritis, peptic ulcer disease and gastric cancers. Fluoroquinolones have been shown to be active against *H. pylori.* For develop new anti-*H. pylori* agents, we have investigated the SAR of a series of *N*-(phenethyl)piperazinyl quinolones for their antimicrobial activity against *H. pylori*. The anti-*H. pylori* activity of synthesized compounds along with commercially available anti-*H. pylori* agents such as metronidazole, and parent quinolones was evaluated by the disc diffusion bioassay. The results indicated that the potency and anti-*H. pylori* activity profile of the quinolones is highly dependent on the type of substituent at N-1 and the structure of phenethyl unit on piperazine ring. Most compounds containing a cyclopropyl at N-1 exhibited good activity against *H. pylori* strains. Among them, ciprofloxacin derivative **13** containing 2-methoxyimino-2-(2-chlorophenyl)ethyl moiety was the most active compound.

Key Words: Fluoroquinolones, 7-piperazinyl quinolones, *Helicobacter pylori*, antibacterial activity.

INTRODUCTION

Helicobacter pylori infection is the main cause of gastritis and gastroduodenal ulcer disease, and is associated with gastric cancer [1, 2]. Hence since 1994, the World Health Organization (WHO) has classified *H. pylori* as a Class 1 carcinogen responsible for its leading role in the development of gastric neoplasia in humans. Thus the eradication of *H. pylori* can significantly reduce the risk of ulcer relapse and may help prevent the lymphoma of gastric mucosa-associated lymphoid tissue (MALT-lymphoma) and other gastric malignancies [3-5]. The recommended therapy consists of a proton pump inhibitor (PPI) and two antibiotics, mainly amoxicillin and clarithromycin, as first-line eradication triple therapy [6,7]. Although this treatment has been shown to be effective in a number of clinical trials, several meta-analysis revealed that the rates of eradication were widely variable (from 70% to 95%), due to increased resistance to antibiotics. Following failure of the eradication by the first-line treatment, a second-line, quadruple therapy using PPI, bismuth salts, metronidazole, and tetracycline is used [8]. This quadruple regimen still fails to eradicate *H. pylori* in approximately 20–30% of the patients [2]. This has also led to the development of alternative (second- or third-line treatment) regimens including other antibacterial agents such as fluoroquinolones for the treatment of patients with resistant *H. pylori* infection [9-13]. Fluoroquinolone antimicrobial agents are widely used for oral treatment of respiratory, gastrointestinal, and urinary tract infections. These antibacterial agents showed *in vitro* activity against *H. pylori* [14, 15]. In contrast to most other anti-infective drugs, quinolones do not kill bacteria by inhibiting a critical cellular process. Rather, they induce the activities of two essential enzymes, DNA gyrase and topoisomerase IV to kill cells by generating high levels of double stranded DNA breaks. DNA gyrase appears to be the primary cellular target and topoisomerase IV seems to be the secondary target for quinolones in Gram-negative bacteria [16, 17]. In *Helicobacter pylori* dual targeting is not possible because these organisms lack genes for topoisomerase IV, as determined by complete genome sequencing [18].

Acquired resistance to ciprofloxacin and other fluoroquinolones already exists in *H. pylori* [19-21]. Recent surveys also suggest that resistance to fluoroquinolones might have increased over the last years in several countries; in France from 3.3% in 1999 to 17.5% in 2003 [22] and in Korea from 0% in 1987 to 21% in 2003 [23]. Accordingly, there is a need for an effective treatment with a new compound having an excellent anti-*H. pylori* activity.

In previous studies we described the synthesis and antibacterial evaluation of 7-piperazinyl quinolones against Gram-positive and Gram-negative bacteria [24, 25]. In the present study, some 7-piperazinyl quinolones **4-25** in which the N-4 hydrogen of piperazinyl group of ciprofloxacin **1**, norfloxacin **2** and enoxacin **3** replaced with various functionalized phenethyl moieties (Fig. **1**) were evaluated *in vitro* against *H. pylori*, and the structure – anti-*Helicobacter* activity relationships were described.

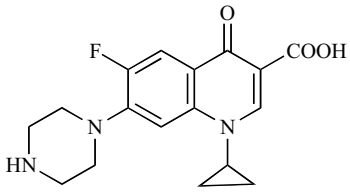
MATERIALS AND METHODS

1. Bacterial Isolates and Culture Conditions

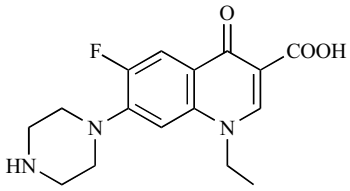
Clinical *H. pylori* strain isolates from antral gastric mucosal biopsy specimens were obtained from the Shariati hos-

*Address correspondence to this author at the Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14174, Iran; Tel: 0098-21-66954708; Fax: 0098-21-66461178;
E-mail: aforoumadi@yahoo.com

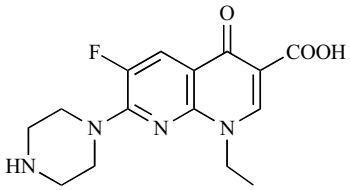
1573-4064/08 $55.00+.00



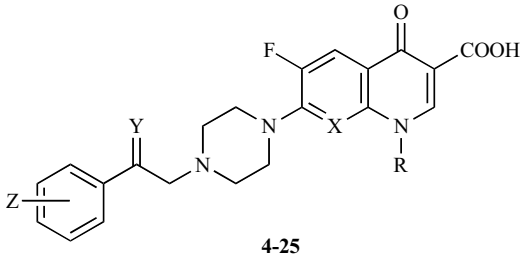
1, Ciprofloxacin



2, Norfloxacin



3, Enoxacin



4-25

R= Cyclopropyl, Ethyl
X= CH, N
Y= O, NOH, NOMe
Z= H, 2-Cl, 4-Cl, 2,4-Cl_2, 2,4-F_2

Fig. (1).

pital (Tehran, Iran). Test strains were precultured on selective Brucella agar (MERK) containing defibrinated blood (7%). The plates were incubated at 37ºC for 3–5 days in an atmosphere of 5% O_2, 15% CO_2, and 80% N_2 in an anaerobic chamber (Hirayama, Tokyo, Japan). To maintain a moist atmosphere, a moist paper towel was placed in the chamber. The identity of bacterial isolates was confirmed by microscopy and positive catalase, oxidase and urease and negative nitrate, H_2S and hippurate hydrolysis reactions. Bacterial strains were stored at -70°C in brain heart infusion broth (BHIB) (Difco, East Molesey, UK) containing 10% (v/v) fetal calf serum (FCS) and 15% (v/v) glycerol until they were used for the experiments.

Before use the media were always preincubated under the same microaerobic conditions for a minimum of 2 h to allow equilibration, and none of the cultures were kept in air for more than 15 min. Given the importance of inoculum homogeneity, cellular viability was controlled microscopically by morphological observation with gram staining, in order to check the proportions of coccoid cells in cultures. Cultures were always used after 48 h of incubation, when they generally did not present coccoid forms. Bacterial growth was taken from the plates and resuspended in sterile saline. The inoculum was prepared to contain about 5×10^7 CFU/ml by adjusting the turbidity of the suspension to match the McFarland no.1 standard.

2. Bacterial Growth Inhibition Assay (Disk Diffusion Method)

Growth inhibition was performed by the filter paper disk diffusion method on selective Brucella agar with 7% of defibrinated horse blood under microaerophilic conditions at 37°C. All compound derivatives were evaluated for their anti-*Helicobacter* activity dissolved in dimethylsulfoxide (DMSO). These compounds were first assayed against metronidazole- resistant and susceptible strains of *H. pylori* at three concentrations (8, 16 and 32 μg/disc) to identify those with little or no activity as leading compounds. Surface of Brucella blood agar plates were inoculated with 100 μl of bacterial suspensions. Glass rods were used to spread the inocula. Blank standard disks (6 mm diameter) were deposited on the plates and impregnated with 10 μl of different dilutions of compounds. The plates were examined after 2-5 days of microaerobic incubation. The susceptibility of *H. pylori* isolates was determined on the basis of the diameter of inhibition zones. The control discs received 10 μl of DMSO. All of the inhibition tests were replicated at least three times. The antibacterial activity was classified as follows: strong response, zone diameter >20 mm; moderate response, zone diameter 16–20 mm; weak response, zone diameter 11–15 mm; and little or no response, zone diameter ≤ 10mm [26].

The most active compounds in the first study were further tested against a broader panel (20 clinical isolates of *H. pylori*) at concentrations of 32, 16, 8, 4, 2, 1 and 0.5 μg/disc.

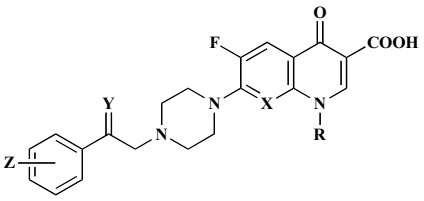
RESULTS

The anti-*Helicobacter pylori* activity of target compounds **4-25** along with commercially available antibacterial such as metronidazole, ciprofloxacin **1**, norfloxacin **2** and enoxacin **3** was evaluated by comparing the inhibition zone diameters determined by the paper disc diffusion bioassay. The compounds were preliminary evaluated against metronidazole-sensitive and metronidazole-resistant *H. pylori* strains at three concentrations (8, 16 and 32 μg/disc). All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced

by title compounds and summarized in Table **1**. Preliminary results indicated that most ciprofloxacin derivatives (compounds **4-8**, **11-13** and **15-17**) exhibit significant activity at the concentrations of 8-32 µg/disc.

In order to assess the potential of title compounds to inhibit different clinical isolates of *H. pylori* growth, the most active compounds **4, 8, 13** and **16** were further tested against a broader panel of *H. pylori* including 20 clinical isolates of

Table 1. Preliminary Evaluation of Compounds 1-25 Against Two Metronidazole Sensitive and Metronidazole Resistant *H. pylori* Strains



Compound	R	X	Y	Z	Metronidazole Sensitive [a] Dose (µg/disc)			Metronidazole Resistant Dose (µg/disc)		
					8	16	32	8	16	32
4	c-Pr	CH	O	2-Cl	27	30	34	22	26	31
5	c-Pr	CH	O	4-Cl	20	30	33	20	21	24
6	c-Pr	CH	O	2,4-Cl_2	17	25	36	6 [b]	6	20
7	c-Pr	CH	O	H	31	35	37	21	23	28
8	c-Pr	CH	NOH	2-Cl	29	30	36	21	25	25
9	c-Pr	CH	NOH	4-Cl	6	6	6	6	6	6
10	c-Pr	CH	NOH	2,4-Cl_2	6	6	6	6	6	6
11	c-Pr	CH	NOH	2,4-F_2	15	17	21	25	30	37
12	c-Pr	CH	NOH	H	25	27	30	17	20	21
13	c-Pr	CH	NOMe	2-Cl	25	35	41	23	35	38
14	c-Pr	CH	NOMe	4-Cl	6	6	6	6	6	6
15	c-Pr	CH	NOMe	2,4-Cl_2	6	26	32	21	25	31
16	c-Pr	CH	NOMe	2,4-F_2	25	30	32	26	30	37
17	c-Pr	CH	NOMe	H	13	15	16	12	17	21
18	Et	CH	NOMe	2-Cl	6	6	6	6	6	6
19	Et	CH	NOMe	4-Cl	6	6	6	6	6	6
20	Et	CH	NOMe	2,4-Cl_2	6	6	6	6	6	6
21	Et	CH	NOMe	2,4-F_2	6	6	6	6	6	6
22	Et	N	NOMe	2-Cl	6	6	6	6	6	6
23	Et	N	NOMe	4-Cl	6	6	6	6	6	6
24	Et	N	NOMe	2,4-Cl_2	6	6	6	6	6	6
25	Et	N	NOMe	2,4-F_2	6	6	6	6	6	6
1	Ciprofloxacin				32	37	42	30	35	38
2	Norfloxacin				25	28	30	20	20	25
3	Enoxacin				8	10	15	6	10	16

[a] Inhibition zone diameters of metronidazole at 8 µg/disc were 18 and 11 mm in metronidazole sensitive and metronidazole resistant strains, respectively.

[b] No inhibition observed (disk diameter was 6 mm).

Table 2. Average of Inhibition Zone Diameters of Selected Compounds at Different Doses Against 20 Clinical *H. pylori* Isolates

Compound	Average of Inhibition Zone Diameter (rang, mm) Dose (µg/disc)						
	0.5	**1**	**2**	**4**	**8**	**16**	**32**
4	6.5 (6-16)	7.1 (6-22)	9.4 (6-27)	12.3 (6-29)	16.8 (6-30)	20.2 (6-34)	24.1 (6-39)
8	6 (6-6)	6 (6-6)	6 (6-6)	9.9 (6-21)	15.6 (6-23)	20.3 (6-29)	24.8 (6-35)
13	7.5 (6-16)	8.6 (6-19)	13.4 (6-30)	19 (6-27)	22.2 (6-33)	25.9 (6-37)	29.1 (6-41)
16	6 (6-6)	7.45 (6-16)	10.4 (6-21)	15.4 (6-25)	17.9 (6-29)	22.2 (12-31)	26.3 (15-39)
Metronidazole				12.0 (6-16)	16.3 (6-20)	17.6 (6-21)	

this microorganism. The antibacterial activities of selected compounds at concentrations of 32, 16, 8, 4, 2, 1 and 0.5 µg/disc against twenty clinical isolates of *H. pylori* are shown as averages of inhibition zone diameters in Table **2**. The averages of inhibition zone diameters indicate that all selected compounds exhibited moderate to good activity against clinical isolates of *H. pylori* at the concentrations of 8-32 µg/disc (average of inhibition zone diameters >15 mm).

DISCUSSION

The 1,4-dihydro-4-oxopyridine-3-carboxylic acid associated with a 5,6-fused aromatic ring is the common chemical feature of quinolone antibacterials. In the resulting bicyclic ring, the 1-, 5-, 6-, 7-, and 8-positions are the major targets of chemical variation but, synthetic efforts for improved potency has been the main focus on 7-position. Both activity spectrum and kinetic profile can be controlled at C-7. The most common substituents are cyclic amino groups, for example piperazine or pyrrolidine rings; other groups have been less successful. Piperazine rings are particularly common (e.g. ciprofloxacin **1**, norfloxacin **2** or enoxacin **3**) and confer potency against Gram-negative bacteria [27-29]. Ciprofloxacin **1**, norfloxacin **2** and enoxacin **3** (Fig. **1**) characterized by having a piperazinee moiety at C-7, which represents a site amenable to significant modification. In addition, a position on the 7-piperazinyl quinolone molecule, where substitutions of bulky groups are permitted, is at the N-4 of piperazine ring. Accordingly, a number of quinolones **4-25** with a 2-oxoethyl or 2-oxyiminoethyl derivatives attached to the piperazine ring at C-7 position were synthesized and evaluated for antibacterial activity against *H. pylori*.

The first information obtained in this study is that most of the ciprofloxacin derivatives exhibit high activity against *H. pylori*. In general, *N*-(2-phenyl-2-oxoethyl)- and *N*-(2-phenyl-2-oxyiminoethyl)- groups are well tolerated in the terms of anti- *H. pylori* activity in ciprofloxacin series.

The anti-*H.pylori* activity profile of the *N*-substituted piperazinyl quinolones is highly dependent on the type of substituent at N-1. Some compounds with a cyclopropyl substituent at N-1 (e.g., **4**, **5**, **7**, **8**, **13**, and **16**) exhibited good activity against metronidazole sensitive and metronidazole resistant *H. pylori* strains at concentrations of 8, 16 and 32 µg/disc (inhibition zone diameter >20 mm). Replacement of cyclopropyl with ethyl at the N-1 position in compounds **18-25** led to a dramatic decrease in activity. In addition, introduction of a nitrogen atom at C-8 position in compounds **22-25** could not improve anti-*H. pylori* activity in N_1-ethyl quinolones.

Among ciprofloxacin series **4-17**, all compounds having carbonyl group on *N*-phenethyl side chain (compounds **4-7**) showed significant activity against *H. pylori* strains. Oxime containing compounds with 4-chloro-substituent (compound **9**) or 2,4-dichloro-substituent (compound **10**) did not show antibacterial activity. Similar to latter compounds, 4-chloro-*O*-methyloxime analog (compound **14**) did not show any growth inhibitory activity. In contrast, *N*-phenethyl ciprofloxacin derivatives containing 2,4-diflouro-substituents exhibited respectable anti-*H. pylori* activity.

Halogens, like chlorine or fluorine, are very useful to modulate the electronic effects on phenyl rings of drugs. Moreover, these atoms may also influence the steric characteristics and the hydrophilic–hydrophobic balance of the molecules. On the other hand, carbonyl related functional groups (ketone, oxime, and *O*-methyloxime) have different steric, electronic, and lipophilic characteristics. Thus, these structural modifications of the *N*-(phenethyl)piperazinyl quinolones were expected to allow modulation of the physical properties of the parent quinolone, while retaining the strong biological activity of the piperazinyl quinolone ciprofloxacin.

Comparison between inhibition zone diameters of ketones **4-7**, oximes **8-12** and *O*-methyl oximes **13-17** in ciprofloxacin series revealed that oximation of ketones diminished the activity, with the exception of 2-chloro- derivative (ketone **4** *vs.* oxime **8**). In addition, *O*-methylation of oximes improved the activity. First approach in the series of *N*-(phenethyl)-piperazinyl quinolones bearing different struc-

tural features on the quinolone ring (N-1 and 8-position) and *N*-(phenethyl)- side chain points out that the *O*-methyl oxime **13** exert more potent *in vitro* antibacterial activity against both metronidazole sensitive and metronidazole resistant *H. pylori* strains. The latter compound that belong to *N*-[2-(2-chlorophenyl)ethyl]ciprofloxacin derivative, also exhibited more significant growth inhibitory activity against 20 clinical isolates of *H. pylori* (Table **2**).

In conclusion, we have identified a series of *N*-substituted piperazinyl quinolones **4-25** in which the N-4 hydrogen of piperazinyl group of ciprofloxacin, norfloxacin and enoxacin replaced with various functionalized phenethyl moieties with *in vitro* antibacterial activity against *H. pylori*. The SAR of this series indicates that both the structure of the phenethyl unit on piperazine ring and the type of N-1 alkyl group dramatically impact anti- *H. pylori* activity. Our data suggested that the effect of changes in the side chain of the 7-piperazinyl ring mainly dependent on substituent at N-1 position.

ACKNOWLEDGEMENTS

This work was supported by grants from the Research Council of Tehran University of Medical sciences and INSF (Iran National Sciences Foundation).

REFERENCES

[1] Manabe, S.; Ishii, K.; Ito, Y. *J. Org. Chem*., **2007**, *72*, 6107.
[2] Gisbert, J. P.; Gisbert, J. L.; Marcos, S.; Moreno-Otero, R.; Pajares, J. M. *Helicobacter*, **2007**, *12*, 68.
[3] Chimenti, F.; Bizzarri, B.; Bolasco, A.; Secci, D.; Chimenti, P.; Carradori, S.; Granese, A.; Rivanera, D.; Lilli, D.; Zicari, A.; Scaltrito, M. M.; Sisto, F. *Bioorg. Med. Chem. Lett*., **2007**, *17*, 3065.
[4] Chimenti, F.; Bizzarri, B.; Bolasco, A.; Secci, D.; Chimenti, P.; Carradori, S.; Granese, A.; Rivanera, D.; Lilli, D.; Scaltrito, M. M.; Brenciaglia, M. I. *Eur. J. Med. Chem.*, **2006**, *41*, 208.
[5] Chimenti, F.; Bizzarri, B.; Manna, F.; Bolasco, A.; Secci, D.; Chimenti, P.; Granese, A.; Rivanera, D.; Lilli, D.; Scaltrito, M. M.; Brenciaglia, M. I. *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 603.
[6] Yoshida, Y.; Barrett, D.; Azami, H.; Morinaga, C.; Matsomoto, Y.; Takasugi, H. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 1897.
[7] Sharara, A. I.; Chaar, H. F.; Racoubian, E.; Moukhachen, O.; Barada, K. A.; Mourad, F. H.; Araj, G. F. *Helicobacter*, **2004**, *9*, 255.
[8] Nishimori, I.; Minakuchi, T.; Kohsaki, T.; Onishi, S.; Takeuchi, H.; Vullo, D.; Scozzafavac, A.; Supuranc, C. T. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 3585.
[9] Bogaerts, P.; Berhin, C.; Nizet, H.; Glupczynski, Y. *Helicobacter*, **2006**, *11*, 441.
[10] Antos, D.; Schneider-Brachert, W.; Bästlein, E.; Hanel, C.; Haferland, C.; Buchner, M.; Meier, E.; Trump, F.; Stolte, M.; Lehn, N.; Bayerdorfferâ, E. *Helicobacter*, **2006**, *11*, 39.
[11] Gisbert, J. P.; Castro-Fernandez, M.; Bermejo, F.; Perez-Aisa, A.; Ducons, J.; Fernandez-Bermejo, M.; Bory, F.; Cosme, A.; Benito, L. M.; Lopez-Rivas, L.; Lamas, E.; Pabon, M.; Olivares, D. *Am. J. Gastroenterol*., **2006**, *101*, 243.
[12] Gisbert, J. P.; Morena, F. *Aliment. Pharmacol. Ther.*, **2006**, *23*, 35.
[13] Wong, W. M.; Gu, Q.; Yee, Y. K.; Fung, F. M. Y.; Tong, T. S. M.; Chan, A. O. O.; Lai, K. C.; Chan, C. K.; Wong, B. C. Y. *Aliment. Pharmacol. Ther.*, **2006**, *23*, 421.
[14] Carbone, M.; Fera, M. T.; Cecchetti, V.; Tabarrini, O.; Losi, E.; Cusumano, V.; Teti, G. *Antimicrob. Agents Chemother.*, **1997**, *41*, 2790.
[15] von Rosenstiel, N.; Adam, D. *Drug*s, **1994**, *47*, 872.
[16] Mitscher, L. A. *Chem. Rev*., **2005**, *105*, 559.
[17] Anderson, V. E.; Osheroff, N. *Curr. Pharmaceut. Des.*, **2001**, *7*, 339.
[18] Hooper, D. C. *Clin. Infect. Dis.*, **2001**, *32*, 9.
[19] Fujimura, S.; Kato, S.; Iinuma, K.; Watanabe, A. *J. Med. Microbiol.*, **2004**, *53*, 1019.
[20] Tankovic, J.; Lascols, C.; Sculo, Q.; Petit, J. C.; Soussy, C. J. *Antimicrob. Agents Chemother.*, **2003**, *47*, 3942.
[21] Boyanova, L.; Mentis, A.; Gubina, M.; Rozynek, E.; Gosciniak, G.; Kalenic, S.; Göral, V.; Kupcinskas, L.; Kantarçeken, B.; Aydin, A.; Archimandritis, A.; Dzierżanowska, D.; Vcev, A.; Ivanova, K.; Marina, M.; Mitov, I.; Petrov, P.; Özden, A.; Popova, M. *Clin. Microbiol. Infect.*, **2002**, *8*, 388.
[22] Cattoir, V.; Nectoux, J.; Lascols, C.; Soussy, C.; Cambau, E., *Helicobacter*, **2005**, *10*, 542.
[23] Kim, J. M.; Kim, J. S.; Kim, N.; Jung, H. C.; Song, I. S. *J. Antimicrob. Chemother.*, **2005**, *56*, 965.
[24] Foroumadi, A. ; Emami, S.; Davood, A.; Moshafi, M. H.; Sharifian, A.; Tabatabaiee, M.; Tarhimi Farimani, H.; Sepehri, G.; Shafiee, A. *Pharmaceut. Sci.*, **1997**, *3*, 559.
[25] Foroumadi, A.; Ghodsi, S.; Emami, S.; Najjari, S.; Samadi, N.; Faramarzi, M. A.; Beikmohammadi, L.; Shirazi, F. H.; Shafiee, A. *Bioorg. Med. Chem. Lett*., **2006**, *16*, 3499.
[26] Mirzaei, J.; Siavoshi, F.; Emami, S.; Safari, F.; Khoshayand, M. R.; Shafiee, A.; Foroumadi, A. *Eur. J. Med. Chem.*, **2007**, In Press: doi: 10.1016/j.ejmech.2007.11.019.
[27] Domagala, J. M. *J. Antimicrob. Chemother.,* **1994**, *33*, 685.
[28] Tillotson, G. S.; Blondeau, J. M. In *Moxifloxacin in Practice*; Adam, D.; Finch, R. G.; Hunter, P. A., Eds.; Maxim Medical, **1999**, pp. 91-101.
[29] Asahina, Y.; Ishizaki, T.; Suzue, S. *Prog. Drug Res.,* **1992**, *38*, 57.

Received: 26 December, 2007 Revised: 31 January, 2008 Accepted: 31 January, 2008